This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 21!

See attached form for additional information.

Interagency Report Control No.:

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. CERTIFICATE NUMBER: 58-R-0003
CUSTOMER NUMBER: 859

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

University Of Florida
P.O. Box 115500
Gainesville, FL 32611

Telephone: (352) -392-9271

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, or experimentation, or held for these purposes. Attach additional sheets if necessary)

FACILITY LOCATIONS (Sites) - See Atached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY **(Attach additional sheets if necessary or use APHIS Form 7023A)**

A. Animals Covered By The Animal Welfare Regulations	B. Number of animal being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not ye used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use o pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals an for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for wh the use of appropriate anesthetic, analgesic, or tranquiliz drugs would have adversely affected the procedures, res or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reasc such drugs were not used must be attached to this report	F. TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs	0	50	170	0	220
5. Cats	0	2	285	0	287
6. Guinea Pigs	0	10	194	0	204
7. Hamsters	0	0	0	0	0
8. Rabbits	0	9	159	90	258
9. Non-human Primates	0	0	40	0	40
10. Sheep	0	1	70	0	71
11. Pigs	0	4	91	0	95
12. Other Farm Animals					
Equine	1	133	69	0	202
13. Other Animals					
Cattle	1	480	14	0	494
Armadillos	0	2	0	0	2
Chinchillas	0	0	50	0	50

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anestetic, analgesic, and tranquilizing drugs, prior to, during, and following actual rese teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and ap Institutional Animal Care and Use Committee (IACUC). **A summary of all such exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary inc brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional Official)

(B)(6) (B)(7)(c)

DATE SIGNED 11/21/08

APHIS FORM 7023 (AUG 91) (Replaces VS FORM 18-23 (OCT 88), which is obsolete.)

NOV 25 2008 NP

This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

CONTINUATION SHEET FOR ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

1. REGISTRATION NO. 58-R-003 CUSTOMER NO. 859

FORM APPROVED OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

University of Florida
PO Box 115500 Gainesville,FL 32611

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use this form.)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic,analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
Deer Mice	0	498	0	0	498
Goats	0	5	7	15	27
Singing Mice	0	78	104	0	182
Voles	0	161	5	0	166
Wild Birds	0	266	0	0	266

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

SIGNATURE OF C.E.O. OR INSTITUTIONAL OFFICIAL	NAME & TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL	DATE SIGNED
(b)(6), (b)(7)c		11/21/08

APHIS FORM 7023A (AUG 91)

(Replaces VS FORM 18-23 (Oct 88), which is obsolete

PART 1 - HEADQUARTERS

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 58-R-0003

2. Number 62 of animals used in this study

3. Species (common name) Rabbits of animals used in the study.

4. Explain the procedure producing pain and/or distress.

   Title: D290; Gene regulation of mammalian DNA viruses

   Please see attached for procedure information.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine the pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

   Please see attached.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency _____ CFR _____

D290; *Gene regulation of mammalian DNA viruses*

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Rabbit pox is a lethal infection in rabbits. The differential pathogenesis caused by RPV and VV is not recapitulated in rodents, thus rabbits are the model system chosen. Analgesics are not indicated in these studies because their affects on the inflammatory and immune responses would hinder the distinctions observed in the two viral diseases. It is critical that the rabbit pox and vaccinia viral diseases be distinguishable. For the screening of recombinant viruses it is also critical for the disease pathways be uninhibited for the detection of virulent viruses.

1. Procedures for restraint of the rabbits will be as follows:
   - Animals will be restrained in a rabbit restrainer (provided by ACS) or by using a clean towel with minimal restraint by an animal handler.
   - The rabbits will be restrained using a rabbit restrainer or with a towel and both hind flanks will be shaved.
   - The rabbits will be gently restrained with a towel and then injected interdermally with 100ul of buffer and the virus injected intradermally in one or two sites using a 25 gauge needle in one or both hind flanks concurrently in Experiment 1 and 3, with animals in Experiment 2 receiving 100ul of buffer and the virus injected intradermally in one to five sites concurrently using a 25 gauge needle in one or both hind flanks.
   - A sterile Implantable Programmable Temperature Transponder chip (Biomedic Data Systems, Inc.) will be injected subcutaneously at the nape of the neck (dorsal cervical region). The chip transmits data for animal identification and body temperature.
   - The rabbits will be gently restrained with a towel.
   - The skin over the nape of the neck will be gently tented away from the back muscles.
   - The tented fur at the nape of the neck will be washed with ethanol.
   - A 2.2 by 14 mm transponder chip will be inserted under the skin by injection via the sterile 14 gauge needle that houses the chip.
   - The skin will be released and physically examined to ensure the chip is placed under the skin and that there is no damage to the skin.
   - A routine physical exam (abdominal palpitation, auscultation of the heart and lungs, assessment of attitude and hydration, etc.) will be performed daily.

2. Collection of Blood (Survival):
   - Rabbits are minimally restrained.
   - The animal is shaved and the skin is cleaned with 70% ethanol.
   - 1-2ml of blood is collected from the lateral saphenous vein with a 25 gauge needle every 2-3 days after virus administration
   - For collection of 10ml blood, 50mg/kg ketamine and 10mg/kg xylazine will be injected into intramuscularly into the biceps femoris muscle or intraperitoneally.
   - Blood will be collected by cardiac puncture using a sterile 22 gauge 1 ½ inch needle attached to a 12cc syringe.
   - 100-150mg/kg body weight of pentobarbital will be injected into the heart after blood has been collected to induce euthanasia.

3. Necropsy and Tissue collection:
    - Upon euthanasia with pentobarbital and the confirmation of successful euthanasia, as determined by the absence of heart beat and respiration, a necropsy of the animal will be performed.
    - Tissue samples from all the organs, as well as lesions on the dermal phase of the animal, will be collected for virus isolation and characterization.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Rabbitpox is a lethal infection in rabbits. The differential pathogenesis caused by RPV and VV is not recapitulated in rodents, thus rabbits are the model system chosen. Analgesics are not indicated in these studies because their affects on the inflammatory and immune responses would hinder the distinctions observed in the two viral diseases.

Column E Explanation

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1. Registration Number: 58-R-0003

2. Number 6 of animals used in this study

3. Species (common name) Rabbits of animals used in the study.

4. Explain the procedure producing pain and/or distress.

   Title: F086; Biochemical and Molecular studies of Myxoma virus-encoded anti-immune and host range proteins.

   Please see attached for procedure information.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine the pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

   Please see attached.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency _____ CFR _____

F086; *Biochemical and Molecular studies of Myxoma virus-encoded anti-immune and host range proteins*

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Our lab studies genes from myxoma virus, a smallpox-like virus that only infects rabbits. These genes allow the virus to make substances that can change the immune response of infected rabbits to the virus, thereby making the virus more or less deadly. To minimize live animal use, the genes are first tested in cell cultures and only when a promising result is found do we then test it in a live rabbit. Many of these genes will match genes with similar function in other important poxviruses, such as smallpox, that infect humans but can not be directly studied in humans or animals. Additionally some of these genes have been found to prevent complications due to surgery in laboratory animals and thus may have a second application in humans.

Myxoma virus causes fatal disease in lab rabbits called myxomatosis. The best way to study the function of a specific gene in this virus is to remove it from the virus (creating what is called a "knock out virus") and then study how the disease progresses compared it to infection with the unaltered ("wild type") virus in the rabbit. Although myxoma virus can infect wild rabbits from North and South America, it does not cause disease in our native rabbits. Myxoma virus only produces the disease myxomatosis in European species of rabbits, which includes the white laboratory rabbit. Therefore, we can only test the progression of this disease using laboratory rabbits. To do so, rabbits will be infected under a shaved area of skin with either the knockout virus. If the virus causes significant disease, the rabbit is humanely killed before the disease becomes too severe. If the virus caused little or no disease, rabbits will be infected with wild type virus 21 days later to determine whether or not infection with the knockout virus provides protection from infection with the wild type virus. In other studies, rabbits infected with the knockout virus will be humanely killed at various times after infection to better determine the effects of the knockout virus on the rabbit.

Objectives: 1. Identify and characterize predicted host range and anti-immune proteins encoded by myxoma virus. 2. Determine the molecular features of the particular gene and protein through cell based studies initially. 3. Once we have an indication of possible function we can test our prediction by monitoring disease in an end point study in rabbits. This will be followed by a histology study in which tissue and blood will be collected at several days post infection and checked for influx of immunological markers and virus concentration.

Research Plan: Every animal study begins with a pathogenesis experiment to determine whether the specific gene deletion has resulted in an attenuated virus. We normally do 3-4 of these types of studies in a single year. The study begins with bringing the rabbits to the infectious disease suite level II facility. Shaving the rabbits at the site of injections. Normally the rabbits are injected subQ with virus on the same day and beginning the next day (day 1) the rabbits are monitored daily by the researcher and the vet techs. For each study there is control animals

infected with wild type and revertent virus. A previously developed scoring system (see the attachment) will be followed and each rabbit is scored daily for a range of parameters including food and water intake, body temperature, weight, appearance and numbers of secondary lesions, conditions of the ears, eyes and noses and liquid and solid waste production. After about day 6 the rabbits are monitored twice daily because myxomatosis starts to become evident. By day 9 onwards infected rabbits are anaesthesized and then the researcher will euthanasize them in order to collect whatever tissues are necessary and any post-morteums that are required. Rabbits injected with an attenuated virus will be monitored for 21 days. If this is the first study with the attenuated virus then the recovered animals are challenged with the wildtype virus to confirm that the animals are protected.

Histology studies: for some knock out viruses, of which we may do 1-2 a year begin the same way. However, the infected animals are normally sacrificed on days 3, 7 and 10 post infection. At these time points the tissue and blood is collected for analysis in the lab.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

I have searched for alternative methods that could be used to minimize pain and distress and have found none that are currently available. The reason our study is a category 3 is that our analyses require infection of rabbit with myxoma virus by subcutaneous route which produce clinical signs of myxomatosis. Late in infection rabbits may develop acute bacterial infection of the conjunctive and upper respiratory tract and these secondary infections have been postulated as a cause of death. Unfortunately, there is no suitable alternative to this infection process in order to establish pathogenesis. However, we will continue to perform periodic searches so that we would be informed if alternatives are developed.

Column E Explanation

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1. Registration Number: 58-R-0003

2. Number 22 of animals used in this study

3. Species (common name) Rabbits of animals used in the study.

4. Explain the procedure producing pain and/or distress.

   Title: 200701022; Antiviral activity of lipid conjugated nucleoside analogs against orthopoxviruses

   Please see attached for procedure information.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine the pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

   Please see attached.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency _____ CFR _____

200701022; *Antiviral activity of lipid conjugated nucleoside analogs against orthopoxviruses*

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Rabbits infected with rabbitpox virus are a unique animal model for smallpox studies because the disease closely mimics the pathology of the human disease, and because rabbitpox virus can be spread between animals in the absence of mechanical or insect vectors. In addition, federal regulations require the use of 2 animal species when using animal data to establish efficacy for human drugs.

To meet the first objective, a set of eight experiments are planned to further refine the dose and dosing regimen that provides optimal protection from both virus spread and morbidity. Each experiment consists of 20 rabbits. There are 2 placebo drug treated RPV or VV infected animals, and 3 groups of 6 rabbits that receive drug per experiment. Previous studies have confirmed that LIP-CDV (lipid-cidofovir conjugate) is able to prevent rabbitpox-induced mortality in infected rabbits and some treatment conditions completely ablate disease. The doses to be used for these experiments, ranging from 1 to 40 mg/kg/day, have been selected based on previous studies in rabbits. The effects of LIP-CDV on RPV spread and titer within the animal will be monitored via blood draws every other day and tissues taken a necropsy.

The second objective is to determine the minimum dose and time of dosing of LIP-CDV to prevent the spread of virus from an infected animal to uninfected cage mates. This will be determined in a set of 6 experiments with each experiment will containing 4 groups of 6 rabbits; the 6 rabbits making up a group will be co-housed in a cage meeting all required guidelines. 2 rabbits will be infected with RPV and receive no LIP-CDV, 4 rabbits will be treated with LIP-CDV at various doses/ dosing schedules to determine the necessary dose and administration of LIP-CDV to prevent disease. The doses will range from 0.2 to 40mg/kg/day based upon the previous experiments that determined effective doses to protect the infected rabbits from disease.

The third objective is to determine if a topical gel is effective in preventing the spread of virus from the site of intradermal injection. This is to be used as a model for an adverse reaction during smallpox vaccination. Two experiments with 20 rabbits per experiment are planned for this objective. Each experiment will have 2 infected untreated animals and 3 groups of 6 rabbits that receive the topical drug up to twice a day. Rabbits will have up to 2ml of water soluble gel placed on the site of inoculation for 1 to 7 days up to twice a day.

The experiments will require minimum manual restraint of the rabbits, shaving of both hind flanks and the intradermal inoculation of 100ul buffer containing the corresponding plaque forming units of virus (0 to 10^8 pfu). Animals will be treated with an oral preparation of LIP-CDV in a minimal volume (i.e., less than 10 ml) or with a topical gel containing LIP-CDV a maximum of two times per day. Rabbits will undergo blood draws and necropsies at euthanasia for tissue samples to determine the effect of LIP-CDV on virus replication.

Following infection, animals will be routinely monitored two times a day (early morning and late afternoon), until or unless symptoms of disease develop. Upon onset of symptoms, the animals will be closely observed four times a day (early morning, mid-day, late afternoon and late evening) for signs of severe disease (as indicated by decreased respiratory rates, dyspnea, open-mouth breathing, severe lung sounds, temperature equal to or exceeding 106°F, anorexia (not to exceed four days), dehydration, and/or morbidity). At this point the animals will be euthanized according to the AVMA Panel on Euthanasia. A set of criteria for scoring the level of disease in the animals has been created and is attached. For placebo animals the expected time to euthanasia is 6-8 days, for treated animals that have no symptoms the experiments will last between 10 and 21 days.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Rabbitpox and vaccinia virus are lethal infections in rabbits. Analgesics are not indicated in these studies because their affects would make it difficult to identify any adverse effects caused by drug treatment. It is critical to be able to identify any potential drug related toxicities during the course of these experiments. Analgesics are not indicated in these studies because of their affects on the inflammatory and immune responses that could possibly hinder the typical clinical observations previously documented in rabbits infected with RPV and VV. It is critical that the disease be observed unhindered.

- Animals are euthanized when signs of severe disease are present and are not allowed to spontaeously die.

Column E Explanation

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1. Registration Number: 58-R-0003

2. Number 15 of animals used in this study

3. Species (common name) Goats of animals used in the study.

4. Explain the procedure producing pain and/or distress.

   Title: 200801274; Proteolytic phenotype and virulence of the Mycoplasma mycoides cluster

   Please see attached for procedure information.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine the pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

   Please see attached.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency _____ CFR _____

200801274; *Proteolytic phenotype and virulence of the Mycoplasma mycoides cluster*

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

We have considered alternatives to the use of goats to study host responses against *Mmm*LC and to study the effect of knocking out protease production on the host response. There is currently no alternative animal model for this disease, and although aspects of the host response can be studied using *in vitro* systems, not enough is currently known about the immune response to this pathogen to proceed to these types of focused studies. At this time there is no substitute for studies using the pathogen in its natural host (goats). Previous results from this laboratory indicate our ability to mimic naturally-occurring infections and achieve our experimental objectives in this species. The information gained from this study will greatly improve our knowledge of infections caused by the *Mycoplasma mycoides* cluster and will be used to further develop preventative strategies for the disease caused by these bacteria. Therefore, the species used in this study stands to benefit from the information gained by our research. We are employing methods to allow us to achieve our experimental objectives with a minimum number of animals by using tissues and cells from the same animal for multiple *in vitro* experiments. In addition we will examine data after the second repetition of the study and will only perform a third repetition if it is required to achieve the statistical power needed to adequately test our hypotheses.

Note that in the initial proposal to the funding agency we discussed performing this study in calves, with goats as an alternative. Cattle are the host of the closely-related *Mmm* Small-Colony bacteria that causes contagious bovine pleuropneumonia. Because *Mmm*LC is an endemic pathogen of goats in the U.S. we subsequently have concluded that goats would be a more appropriate model with less overall risk for severe disease in infected animals. Based on our previous work, the objectives can be achieved in goats and we will therefore perform the studies in this species.

Management of goats prior to transport to ACS facilities – Goat kids aged 3 to 6 months will be purchased through ACS. The goats will be fully weaned for at least 2 weeks prior to purchase and have no history of clinical disease. They will be treated appropriately for external and internal parasites and be vaccinated against *Clostridium perfringens* type C and D and tetanus at least 2 weeks prior to shipping. Goats will be screened for *Mmm*LC infection status prior to purchase by collection of a blood sample to assess serum antibody titers to *Mmm*LC and nasopharyngeal swabs to assess infection status by culture and polymerase chain reaction (PCR). These samples can be collected at the same time that samples are collected to assess Q-fever status. A physical examination of goats by a veterinarian will be performed at that time and only healthy animals selected for purchase. If there is evidence of mycoplasmal infection in the source herd goats will be treated with an anti-mycoplasmal antibiotic (such as short-acting tetracycline, tylan or florfenicol) prior to shipping; this antibiotic will be selected in consultation between the ACS veterinarian and the investigator.

Management of goats during the conditioning period – Goats will be transported to a Biosafety Level 2 (BSL2) containment facility, Progress Center, Alachua, where they will be housed in groups of 3. They will be acclimated for at least 7 days prior to starting on the study. Goats will be weighed upon arrival.

They will be treated with ceftiofur sodium or ceftiofur hydrochloride (1.1 mg/kg subcutaneously (SQ) once daily for 3 days) upon arrival to help prevent transport-related respiratory disease and to reduce the risk of non-mycoplasmal respiratory infections. Goats will be checked for infection with intestinal parasites or coccidia by fecal examination and, if indicated, treated appropriately through consultation with the ACS veterinarian. Blood samples, nasopharyngeal and oropharyngeal swabs will be collected to confirm the mycoplasma-free status of the goats after arrival (see below). A complete physical examination will be performed on each animal by a veterinarian or other trained personnel upon arrival and then daily.

Experimental design and management of goats during the study period– There will be 3 infection groups; group 1 will be infected with the *Mmm*LC GM12 strain, group 2 will be infected with an *Mmm*LC GM12 mutant where the gene for protease production has been disrupted, and group 3 will be a sham-infected control group. The study will be conducted in repetitions of 3 animals per group, with 2 (n=6 per group total) or 3 (n=9 per group total) repetitions. The three groups (two infection groups and the control group) will be housed in separate rooms and will be assigned separate equipment. BSL-2 containment practices will be followed regarding all equipment and personnel contacting goats to prevent cross-infection between groups or escape of the infectious agent from the rooms.

A veterinarian or other trained personnel will perform a complete physical examination on each goat every day. Body weight will be recorded on day 0 and then weekly. Goats that develop clinical signs of illness will be weighed daily. Samples will be collected during the study period as described below. The protocol for monitoring goats that develop clinical disease and the criteria for euthanasia are described in 20.5.1. Goats that do not develop signs of illness requiring euthanasia will be euthanized at the end of the study as described in 19.1.1a.

A complete necropsy will be performed and appropriate tissues collected for our outcomes of interest. Our experiments require that we remove large tissue samples immediately after euthanasia. Portions of these will be transported to our BSL-2 lab on the third floor of the Basic Science Building for processing. Tissues will weigh a maximum of 3 kg and will be placed on ice in sealed sterile plastic bags following harvest from the animal. Bags will be transported to the lab in a closed secondary container. Any tissue not used will be sealed in biohazard bags and returned to ACS for incineration.

Details of specific procedures

Blood samples – During the 21-day infection period we need to collect blood at frequent intervals to enable us to detect any temporal differences in cellular and humoral immune response between groups. Blood will also be used in monitoring the health and infection status of the animals. Blood will be collected every 3 days during the infection period and will also be collected upon arrival at ACS to confirm that goats have low serum antibody levels to *Mmm*LC.

* Calculations are based on a circulating blood volume of 70ml/kg and a minimum body weight of 10 kg; we anticipate that the majority of kids will weigh more than this and we will therefore need to collect a smaller proportion of the circulating blood volume.

** At 21 days, approximately 10% of the circulating blood volume will be collected immediately prior to euthanasia.

Over any given 14 day period throughout the study, a cumulative maximum of 18% of the circulating blood volume will be collected. Hematological parameters (hematocrit and serum albumin concentration) will be monitored on days 3, 6 and 15 of the study period. We do not anticipate any problems associated with blood collection, but if any animal does develop evidence of blood-loss-associated anemia or hypoalbuminemia, then no further blood samples will be collected until those parameters return to normal, or until immediately prior to euthanasia (whichever is first). If such signs are noted we will also consult with the ACS veterinarians.

Blood samples will be collected from the jugular vein using standard procedure. An area over both jugular veins will be clipped upon arrival at ACS and repeated as necessary throughout the study. A veterinarian or other trained personnel will collect samples by jugular venipuncture with 1-inch 20-gauge needles using aseptic technique. Goats will be manually restrained in their normal housing area for sample collection. This technique is rapid, easy to perform and causes minimum distress to the animal (restraint and a needle prick).

Upper respiratory tract swabs - To monitor for nasal shedding of *MmmLC* and other pathogens in respiratory secretions, nasopharyngeal swabs will be collected on arrival at the ACS facility and at various times during the experiments (listed under study timeline, above). To obtain swabs, goats will be manually restrained in their normal housing area. Obvious dirt and debris will be cleaned from the external nares by wiping with a gauze sponge soaked in sterile phosphate buffered saline (PBS). A sterile 5-inch cotton-tip swab will be inserted into a nostril, the nasopharyngeal epithelium wiped briefly (1-2 seconds) and the swab withdrawn. This procedure normally takes less than a minute and causes minimal distress to the animal. At arrival and on days 0, 3 and 12 of the study we will also collect a swab of the oropharynx (palatine tonsil region) to monitor for mycoplasma colonization. This is because mycoplasmal colonization of the upper respiratory tract can be restricted to the tonsilar region without nasal shedding. To obtain oral swabs, goats will be restrained in their normal housing area and an oral speculum placed in the mouth over the tongue, through which a guarded swab will be used to briefly swab the oropharynx. The placement of an oral speculum to depress the back of the tongue is a routine procedure used in large animal medicine, and although ruminants typically resist placement of a speculum (or any other object) in their mouth, it does not cause any physical injury. The procedure to obtain oropharyngeal swabs is very brief, less than a minute.

Inoculation - Goats will be inoculated using a combined oral and nasal route. They will receive a total dose of approximately 5×10^7 colony forming units (CFU) of *Mmm*LC GM12 in growth media (group 1), an *Mmm*LC mutant (group 2), or an equivalent volume of sterile carrier (group 3). The dose was optimized during a previous infection study that utilized *Mmm*LC GM12. Half of the inoculum will administered orally by mixing broth culture (groups 1 and 2) or sterile growth media (group 3) in 100-ml of sterile goat milk replacer and syringe-feeding this to the goats. The second half of the inoculum will be washed by centrifugation to remove growth media and resuspended in 2-ml of sterile, endotoxin-free, isotonic saline (groups 1 and 2). This will be administered intranasally; the control group (group 3)

will receive sterile, endotoxin-free isotonic saline. The combined intranasal/oral route mimics naturally-occurring infection by establishing colonization of the upper respiratory tract, and we have previously demonstrated that the oral route of inoculation results in infection and clinical disease in young goats.

Goats will be manually restrained in their normal housing area for inoculation. The inoculation procedures are not painful. Intranasal administration of a 2-ml volume may result in a brief period of sneezing immediately after administration. Inoculation does not present a risk to personnel. All personnel contacting goats wear protective personal protective equipment (PPE) including a surgical mask. We expect that minimal aerosol would be created even if a goat does sneeze during the procedure, and the mask affords protection against any such aerosol. Importantly, mycoplasmas are host-specific (in mammals) and *Mmm*LC does not cause disease in humans.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Unfortunately, a major part of the disease associated with MmmLC is caused by the response of the immune system to infection. Alleviation of clinical signs of respiratory disease in ruminants is usually achieved through the use of antibiotics (to eliminate the pathogen) and anti-inflammatory drugs, which act by suppressing the inflammatory component of the immune response. However, a major purpose of these studies is to assess the development of immune responses to infection. Thus, the use of antibiotics or anti-inflammatory drugs would negate the usefulness of our study. Suitable alternatives for the long-term relief of pain or inflammation in goats are not available. Goats will not be treated for mild clinical signs of MmmLC-associated disease. Although we do not expect severe clinical disease, we have established strict criteria for timely intervening euthanasia if animals do develop more severe disease (discussed in 20.5.1). Additionally, if the veterinarian associated with this study or any ACS veterinarian deems that an animal requires pain relief for any reason, the animal will be euthanized immediately.